PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P035883WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/004775	International filing date *(day/month/year)* 10.11.2004	Priority date *(day/month/year)* 10.11.2003
International Patent Classification (IPC) or national classification and IPC A61K38/16, A61K39/106, A61K39/40, G01N33/50, A61P31/00		
Applicant UNIVERSITY OF KENT et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.09.2005	15.02.2006
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004775

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-27 as originally filed

Sequence listings part of the description, Pages

1-15 received on 12.01.2005 with letter of 10.01.2005

Claims, Numbers

1-37 received on 05.09.2005 with letter of 31.08.2005

Drawings, Sheets

1/9-9/9 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-39 as originally filed
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004775

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10,36 (completely); 1-5,20,29,30,35-37 (partially)

because:

☒ the said international application, or the said claims Nos. 1-5,20,29,30,35-37 with regard to industrial applicability relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 10,36 (completely); 35 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-9,11,12,24,28-33
	No:	Claims	1-5,13-23,25-27,34,35,37
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-9,11-35,37
Industrial applicability (IA)	Yes:	Claims	6-9,11-19,21-28,31-34
	No:	Claims	1-5,20,29,30,35,37

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item III.

1. Claims 1-5,20,29,30,35,37 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

2. Present claims 10 and 36 relate to a compound defined by reference to the following parameter:
P1: an anti-idiotypic antibody that binds to an antibody which recognizes a certain domain of LuxR.
The use of this parameter in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameter the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, no search has been carried out for subject-matter of claims 12 and 38.

3. Further, present claim 35 relates to an extremely large number of possible methods. In fact, the claims contain so many options, that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely for a method as detailed in Example 5 of the application.

Re Item V.

The following documents are referred to in this communication:

D1 : WO 03/087145 A (AFFINIUM PHARMACEUTICALS, INC) 23 October 2003 (2003-10-23)

D3 : DEVINE J H ET AL: "Nucleotide sequence of the luxR and luxI genes and structure of the primary regulatory region of the lux regulon of Vibrio fischeri ATCC 7744" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 27, 1988, pages 837-842, XP002152246 ISSN: 0006-2960

The applicant's comments, filed with the letter dated 31.08.2005, have been considered before issuing the present report.

1. Amendments (Art 19(2),34(2)(b), PCT)

The amendments filed with the letter dated 31.08.2005 do not add any subject-matter which extends beyond the contents of the application as filed.

2. Novelty and inventive step (Art. 33(2)(3), PCT)

2.1 The application relates to methods for regulating quorum sensing in bacteria by inhibiting the signalling through LuxR (lux regulator) or a large number of homologues thereof (e.g. SdiA). Antibodies against certain domains of LuxR or its homologues for use as a medicament and for sensitising antibiotic resistant bacteria are claimed as well as screening methods for LuxR antibodies and pharmaceutical compositions, vaccines and kits comprising LuxR, LuxR homologues, LuxR nucleic acids or LuxR antibodies.

2.2 **D1** discloses the *E.coli* homologue of RhlR and LasR, wherein the corresponding gene is designated SdiA, and methods for inhibiting RhlR and LasR for treating drug resistant bacterial infections (p. 37, l. 15-32). According to the definition of LuxR homologues of the application, RhlR and LasR are also considered LuxR homologues. The use of specific antibodies for blocking RhlR and LasR and thereby treating bacterial infections as well as vaccines and pharmaceutical compositions comprising the same are described (p. 88, l. 8-18; p. 29, l. 26-28; p. 108, l. 13-15; p. 111, l. 15-19). Although the blocking of RhlR and LasR by antibodies is not explicitly termed a "method of regulating quorum sensing", it is obvious from the description (p. 37, l. 23-25), that RhlR and LasR are two regulators of quorum sensing in *Pseudomonas aeruginosa* and that consequently the blockage of said receptor proteins by antibodies is a "regulation of quorum sensing".
Hence, subject-matter of claims 1-5,13-23,25-27,34,35 and 37 lacks novelty in view of **D1** and subject-matter of claim 28 seems obvious in view of information on page 37, lines 23-25 and lines 30-31 (Art. 33(2)(3), PCT).

2.3 **D3** discloses the nucleic acid and amino acid sequence of LuxR obtained from *Vibrio fischeri* ATCC 7744.
In light of **D3**, subject-matter of claims 34 and 37 is obvious and thus not in agreement with Art. 33(3), PCT.

2.4 The present application generally claims methods for regulating quorum sensing in bacteria comprising modulating the activation of LuxR or a large number of homologues thereof by use of antibodies which block the binding of a signalling molecule to LuxR or a homologue thereof (p. 3, l. 21-7). It is however doubtful that the method has the same technical effect in <u>all</u> bacteria and on <u>all</u> mentioned LuxR homologues, since it is e.g. not evident that the bacterial membranes of all other bacteria may have the same permeability for said antibodies as compared to the *Vibrio fischeri* strain used in example 4 of the present application. In case the permeability for antibodies is reduced in other bacterial strains as compared to *Vibrio fischeri*, it is likely that said antibodies <u>will have no effect in the modulation of quorum sensing</u>.
Since a blocking effect on luminescence (as read out for inhibition of quorum signalling) has only been shown for an anti-LuxR antiserum but not for a

representative spectrum of antibodies against LuxR homologues, the problem of providing a method for regulating quorum sensing comprising modulating the activation of LuxR or a large amount of homologues thereof is considered as not having been solved. Hence, all claims relating to homologues of LuxR homologues (claims 1-35 and 37) cannot be regarded as involving an inventive step (Art. 33(3), PCT).

Re Item VIII.

3. Clarity (Art. 5, 6, PCT)

3.1 The present wording of claim 1 is ambiguous in the sense that it refers to a method involving an antibody, which might be directed either against LuxR (or one of its homologues) or against a signalling molecule (the latter is not supported by the application).

3.2 At least in the regional phase before the EPO, claims 19, 21-24 will be regarded as not clear, since they relate to mechanisms of diseases instead of concrete diseases as mentioned, e.g., in claim 28.

CLAIMS

1. A method of regulating quorum sensing in bacteria comprising modulating the activation by a signalling molecule of LuxR or a homologue thereof, wherein the binding of an antibody prevents LuxR or homologue of LuxR from being activated by its signalling molecule.

2. A method according to claim 1 wherein said bacteria are Gram negative.

3. A method according to claim 1 or claim 2 wherein said homologue of LuxR is selected from the list consisting of AhlR, AhyR, AsaR, BafR, BisR, BpsR, BviR, CarR, CepR, CerR, CinR, CsaR, CviR, EagR, EcbR, EchR, EsaR, ExpR, HalR, LasR, Mll8752, MupR, PcoR, PhzR, PmlR, PpuR, PsmR, PsyR, RaiR, RhiR, RhlR, SdiA, SdiR, SmaR, SolR, SpnR, SprR, SwrR, TraR, TriR, TrlR, TrnR, VanR, VsmR, Y4qH, YenR, YpeR, YpsR, YruR, YtbR and YukR.

4. A method according to any of claims 1 to 3 wherein the signalling molecule is a N-acylated homoserine lactone.

5. A method according to any previous claim wherein said antibody is a monoclonal antibody.

6. An antibody that immunoreacts with LuxR or a homologue of LuxR in the region between amino acid residues 19 and 80 of SEQ ID NO: 5.

7. An antibody according to claim 6 wherein said antibody immunoreacts with LuxR or a homologue of LuxR in the region between amino acid residues 19 and 31 of SEQ ID NO: 5.

8. An antibody which immunoreacts with the sequence TCNNNKDINQC.

9. An antibody which immunoreacts with LuxR or a homologue of LuxR between the negative regulation domain and the autoinducer-binding domain.

10. An anti-idiotypic antibody that binds to an antibody according to any one of claims 6 to 9.

11. An antibody according to any of claims 6 to 10 conjugated to a detectable label.

12. An antibody according to claim 11 wherein said label is a radioisotope, a fluorescent molecule, a heavy metal molecule or an enzyme.

13. A pharmaceutical composition comprising LuxR, a homologue of LuxR, a fragment of LuxR, a fragment of a homologue of LuxR or a nucleic acid encoding one of these polypeptides, or an antibody according to any one of claims 6 to 12.

14. A vaccine composition comprising LuxR, a homologue of LuxR, a fragment of LuxR, a fragment of a homologue of LuxR, a nucleic acid encoding one of these molecules or a quorum sensing signalling molecule.

15. A vaccine composition according to claim 14 wherein said quorum sensing signalling molecule is a N-acylated homoserine lactone.

16. A vaccine composition according to claim 14 or claim 15 further comprising a pharmaceutically acceptable diluent or carrier.

17. A vaccine composition according to any one of claims 14 to 16, further comprising an adjuvant.

18. LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, an antibody according to any one of claims 6 to 12, a pharmaceutical composition according to claim 13, or a vaccine composition according to claims 14 to 17 for use as a medicament.

19. Use of LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, an antibody according to any one of claims 6 to 12, a pharmaceutical composition according to claim 13, or a vaccine composition according to claims 14 to 17 in the manufacture of a medicament for the treatment of disease in which quorum sensing is implicated.

20. Use of LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, a ligand, an antibody according to any one of claims 6 to 12, a pharmaceutical composition according to claim 13, or a vaccine composition according to claims 14 to 17 for sensitising an antibiotic resistant bacterium to an antibiotic.

21. Use of LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, a ligand, an antibody according to any one of claims 6 to 12, a pharmaceutical composition according to claim 13, or a vaccine composition according to claims 14 to 17 in the manufacture of a medicament for sensitising an antibiotic resistant bacterium to an antibiotic.

22. Use of LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, a ligand, an antibody according to any one of claims 6 to 12, a pharmaceutical composition according to claim 13, or a vaccine composition according to claims 14 to 17 in the manufacture of a medicament for the treatment of a disease in which quorum sensing is implicated wherein the patient suffering from that disease is refractive to antibiotic therapy.

23. Use of LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, a ligand, an antibody according to any one of claims 6 to 12, a pharmaceutical composition according to claim 13, or a vaccine composition according to claims 14 to 17 in the manufacture of a medicament for the treatment of a disease in which quorum sensing is implicated wherein the medicament is administered in conjunction with an antibiotic.

24. Use of an antibiotic in the manufacture of a medicament for the treatment of a disease in which quorum sensing is implicated wherein the subject being treated is pre-administered with a pharmaceutical composition or vaccine according to the invention.

25. The use of claim 19 wherein disease is caused by *Vibrio salmonicida, Aeromonas hydrophila, Burkholderia ambifaria, Burkholderia pseudomallei, Burkholderia mallei, Burkholderia stabilis, Burkholderia vietnamiensis, Burkholderia multivorans, Escherichia coli, Serratia marcescens, Salmonella typhi, Brucella suis, Brucella melitensis, Yersinia ruckeri, Hafnia alvei, Shigella flexneri, Serratia liquefaciens, Enterococcus faecalis, Pseudomonas aeruginosa, Burkholderia cepacia, Pseudomonas fluorescens, Providencia stuartii, Klebsiella aerogenes, Yersinia pestis, Yersinia enterocolitica* or *Yersinia pseudotuberculosis.*

26. The use according to any one of claims 19 to 25 wherein said disease is Crohn's disease or Cystic Fibrosis, cellulites and ecthyma, Glanders, melioidosis, meningitis, septicaemia, pneumonia, enteric infections and urinary tract infections, food poisoning, chest infections, typhoid fever, Malta disease, blood stream infections, shigellosis, salmonelliosis, black death and gastroenteritis, hitra disease in Atlantic salmon, haemmorrhagic septicaemia in marine fish, spontaneous abortion in pigs and sheep, red mouth disease in rainbow trout, and cranial and eye lesions in fish.

27. The use according to any one of claims 20-24 wherein said antibiotic is erythromycin A, rifampin, tetracycline, chloramphenicol, norfloxacin, nalidixic acid or penicillin G.

28. Use of a method according to any one of claims 1 to 5 for the inhibition of biofilms.

29. A method of detection of quorum sensing bacteria comprising;

(i) probing a sample of bacteria with a labelled antibody according to claim 11 or claim 12, and

(ii) detecting the presence of antibody attached to bacteria.

30. A method of detection of quorum sensing bacteria comprising;

(i) probing a sample of bacteria with a first antibody according to any one of claims 6 to 9,

(ii) probing said first antibody with a second, labelled antibody, and

(iii) detecting the presence of the second antibody attached to bacteria.

31. A method of detecting antibodies specific for LuxR or a homologue thereof comprising:

(i) probing a sample of serum with whole bacterial cells expressing whole or a fragment of LuxR or a homologue thereof,

(ii) probing the bacteria/antibody complex with a second, labelled antibody, and

(iii) detecting the presence of the second antibody attached to the bacteria/first antibody complex.

32. A method of detecting antibodies specific for LuxR or a homologue thereof comprising:

(i) probing a sample of serum with purified LuxR or a fragment or homologue thereof,

(ii) probing the bacterial protein/antibody complex with a second, labelled antibody, and

(iii) detecting the presence of the second antibody attached to the bacteria/first antibody complex.

33. A kit comprising an antibody according to any one of claims 6 to 12.

34. A kit comprising a fragment of LuxR or a fragment of a homologue of LuxR for the detection of antibodies thereto.

35. A method of inhibiting quorum sensing comprising sequestering quorum sensing signal molecules.

36. Use of an antibody according to claim 11 in the method of claim 35.

37. A kit comprising for simultaneous, separate or sequential use (i) LuxR, a homologue of LuxR, a fragment of LuxR or a homologue of LuxR, a nucleic acid encoding one of these polypeptides, an antibody, a pharmaceutical composition or a vaccine composition according to the invention and (ii) an antibiotic.